Ann Med Res 2021;28(5):1068-72

Annals of Medical Research

DOI: 10.5455/annalsmedres.2021.01.022

Original Article

Does intravitreal injection of aflibercept affect the corneal endothelium?

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Abstract

Aim: To evaluate the effect of intravitreal aflibercept injections on the corneal endothelium in patients with exudative age-related macular degeneration (AMD), diabetic macular edema (DME), and central retinal vascular occlusion (CRVO).

Materials and Methods: The study included 50 eyes of 44 patients who underwent three consecutive intravitreal aflibercept injections with the diagnosis of AMD, DME or CRVO. All patients underwent complete ophthalmological examination and specular microscopy at pre-injection and at one month after the third injection. The pre-injection and post-injection values of endothelial cell density (CD), coefficient of variation (CoV), hexagonality (Hex), central corneal thickness (CCT), and intraocular pressure (IOP) were compared statistically.

Results: The mean age of the patients was 71.09 ± 5.6 (40–87) years. Thirty-six eyes had exudative AMD, 10 had DME and 4 had CRVO. There were no significant differences in CD, CoV, Hex, CCT, and IOP between the pre-injection and post-injection measurements. However, in patients with AMD, the mean pre-injection CD was 2439.71 ± 476.16 cells/mm^2 and the mean post-injection CD was 2286.18 ± 427.24 cells/mm^2. CD values were decreased in the AMD group, but this difference was not statistically significant ($p = 0.061$). In patients with AMD, the mean pre-injection and post-injection CCT values were 536.1 ± 28.4 µm and 542.5 ± 32.9 µm, respectively, and the difference was statistically significant ($p = 0.04$).

Conclusion: Intravitreal aflibercept injection had no manifest devastating effects on the corneal endothelium. However, the decreasing number of endothelial cells with age may be susceptible to aflibercept injection.

Keywords: Aflibercept; age-related macular degeneration; central corneal thickness; corneal endothelium; diabetic macular edema

INTRODUCTION

Intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents are widely used for the treatment of age-related macular degeneration (AMD) and retinal vascular diseases [diabetic macular edema (DME) and central retinal vein occlusion (CRVO)] (1-5). Currently, bevacizumab, ranibizumab, and aflibercept are the available anti-VEGF agents. The most common side effect of intravitreal anti-VEGF injection is acute transient elevation of intraocular pressure (IOP) immediately after the injection, whereas the most destructive complications are endophthalmitis and rhegmatogenous retinal detachment (6-8).

Most patients receiving anti-VEGF treatment belong to the elderly age group and tend to have senile cataracts. In addition, patients receiving anti-VEGF agents for DME could have an increased incidence of diabetic cataracts (7). Because these patients are likely to receive cataract surgery in the near future, it is important to know the number and function of corneal endothelial cells.

Some recent studies suggested that ranibizumab and bevacizumab might decrease retina pigment epithelium (RPE) barrier function by affecting the tight junctions (9,10). The experimental animal studies showed that intravitreal injection provided a sufficient amount of anti-VEGFs in the anterior chamber (11,12,13) and VEGF receptors were found in corneal endothelial cells (12,14). Therefore, we thought that anti-VEGF agents might affect the tight junctions between corneal endothelial cells and disturb the function of corneal endothelium with a similar mechanism to the RPE.

In this study, we aimed to investigate the effect of intravitreal aflibercept on the corneal endothelial cell morphology in patients with AMD, DME, and CRVO.

MATERIALS and METHODS

In this study, the records of the patients who had received an intravitreal injection of aflibercept (Eylea; Regeneron, Tarrytown, NY) for naive AMD, DME or CRVO between

Received: 07.01.2021 **Accepted:** 17.03.2021 **Available online:** 26.05.2021
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January 2016 and December 2017 were reviewed. Patients who had received three monthly of intravitreal aflibercept (0.05 mL, 2 mg) were included in the study. The study was approved by the hospital ethics committee (2011-KAEK-25 2019/02-11) and the authors adhered to the tenets of the Declaration of Helsinki in all protocols.

The study included only the naive AMD, DME or CRVO patients. The exclusion criteria were as follows: use of contact lens, a history of vitreoretinal surgery, and the presence of corneal disease or glaucoma. Patients who underwent ocular surgery or laser photocoagulation three months before or between injections were excluded from the study.

All subjects underwent a complete ophthalmologic examination, which included testing for visual acuity, biomicroscopy, fundoscopy, non-contact tonometry, and corneal pachymeter (CT.1P, Topcon, Japan). Besides that optical coherence tomography (OCT, Optovue® iVue spectral-domain OCT), and specular microscopy (NSP-9900, NonconRobo, Konan, Japan) were applied to all patients before the first injection and one month after the third injection (Figure 1A and B). On initial examination, fundus fluorescein angiography was performed in all patients.

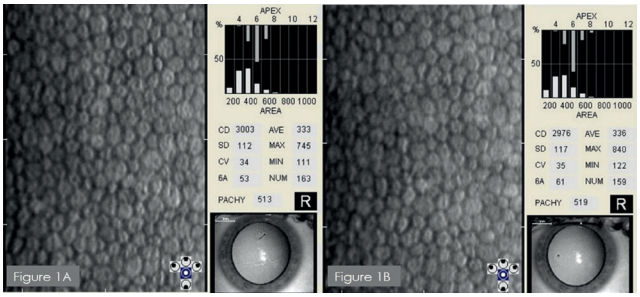

Figure 1. A: Corneal endothelial image by specular microscopy of a patient with diabetic macular edema before the injection. B: Specular microscobic images of the same patient after three aflibercept injections

All injections were administered after topical anesthesia with proparacaine (Alcaine; Alcon). Povidone iodine 10% was applied to the eyelids and periocular skin and eyes were covered with a sterile ophthalmic drape. Povidone iodine at 5% was dripped into the conjunctival fornix. Aflibercept (2 mg/0.05 mL) was injected intravitreally 3.5 mm away from the limbus into the superior and temporal quadrant using a 30-gauge (G) needle. Post-injection light perception was controlled. After the injection, the eye was closed with a bandage for four hours, followed by the administration of topical moxifloxacin hydrochloride five times a day, for five days.

In this study, non-contact specular microscopy (NSP-9900, Konan, Japan) was used. Three measurements were made from the center of the cornea in each patient. All patients were asked to look straight ahead at the fixation targets of the instruments to obtain the images of the similar corneal regions. The automated cell bordering procedure was applied to identify the cell domains on each image. To apply this, a boxed area (region of interest, ROI) approximately in the middle of the image first defined and a filtered image file was obtained. At least 100 adjacent cells were analyzed by the automated methods with Konan CellChek software. Therefore, specular microscopy automatically evaluated endothelial cell density (CD), coefficient of variation (CoV), and cell hexagonality percentage (pleomorphism index; Hex).

The pre-injection and post-injection values of CD, CoV, Hex, CCT, and IOP were compared statistically. SPSS version 22.0 program (SPSS Inc., Chicago, IL) was used for all statistical analyzes. A p value of <0.05 was considered statistically significant. A Mann–Whitney U test was used for analyzing quantitative-dependent data. Paired sample t-test and Wilcoxon test were used for analyzing quantitative-dependent data.

RESULTS

Fifty eyes of 44 patients (18 women and 26 men), with a mean age of 71.09 ± 5.6 (40–87) years, were included in the study. Thirty-six eyes had exudative AMD, 10 eyes had DME and 4 eyes had CRVO. Twenty-two eyes (44.0 %) were pseudophakic, whereas 28 were phakic during the intravitreal aflibercept treatment. All pseudophakic eyes had an intact posterior capsule. The demographic and clinical features of patients are listed in Table 1.

Table 1. Demographic and clinical features of the patients

Characteristics	Eyes (n = 50)
Mean age	71.09 ± 5.6
Gender	
Female	18
Male	26
Diagnosis	
AMD	36
DME	10
CRVO	4
Lens Status	
Phakic	28
Pseudophakic	22

Age-related macular degeneration; AMD, diabetic macular edema; DME, central retinal vein occlusion; CRVO

There was no statistically significant difference between the mean CCT values measured at pre-injection and post-injections (534 ± 29.6 µm and 543 ± 33.8 µm, respectively).

Pre-injection and post-injection values of the mean CD were decreased from 2565.0 ± 461.3 cells/mm^2 to 2500.0 ± 447.7 cells/mm^2, but this difference was not statistically significant (p = 0.081). The mean CoV values were increased from 45.0 ± 6.2% to 46.0 ± 8.7% after three injections, although this was not statistically significant (p = 0.296). Similarly, there was no statistically significant difference between the pre-injection and post-injection values of the mean Hex (p = 0.930).

The mean IOP was 15.08 ± 3.17 mmHg before the first injection and 14.9 ± 2.7 mmHg after the third injection (p = 0.655). Table 2 presents the mean CD, CoV, Hex, CCT, and IOP values of all patients at pre-injection and post-injection.

Table 2. Effects of intravitreal aflibercept injection on CD, CoV, Hex, CCT, and IOP values

Parameters	Pre-injection (mean ± SD)	Post-injection (mean ± SD)	p value
Central Corneal Thickness (µm)	534.5 ± 29.6	543.50 ± 33.8	0.081
Intraocular Pressure (mmHg)	15.08 ± 3.17	14.9 ± 2.7	0.665
Cell Density (cells/mm^2)	2565.0 ± 461.3	2500.0 ± 447.7	0.081
Cell Variation (%)	45.0 ± 6.2	46.0 ± 8.7	0.296
Hexagonality (%)	42.5 ± 8.7	44.5 ± 6.7	0.930

IOP; Intraocular Pressure, SD; Standart Deviation

Patients were classified into two groups according to their lens status. The abovementioned values were analyzed in the pseudophakic and phakic groups. There was no statistically significant difference in pre- and post-injection values for all parameters between the pseudophakic and phakic groups.

In addition, patients were classified into two groups based on their diagnosis. Because only 4 eyes had CRVO, statistical analysis could not be performed on these patients. However, all the parameters were analyzed in the DME and AMD groups individually (Table 3). In the DME group, there were no statistically significant differences between the pre-injection and post-injection values. In the AMD group, the mean pre-injection CD was 2439.71 ± 476.16 cells/mm^2 and the mean post-injection CD was 2286.18 ± 427.24 cells/mm^2, this decrease was not statistically significant (p = 0.061). The mean pre-injection and post-injection CCT values were 536.1 ± 28.4 µm and 542.5 ± 32.9 µm, respectively (p = 0.042). Except for the CCT values, other specular, and IOP values did not show statistically significant differences after three injections (Table 3).

Table 3. Effects of intravitreal aflibercept injection on CD, CoV, Hex, CCT, and IOP values

	Pre-injection	Post-injection	p value
AMD (n = 36 eyes) Mean age, 73.1 years			
Mean Cell Density (cells/mm^2)	2439.71 ± 476.16	2286.18 ± 427.24	0.061
Mean Central Corneal Thickness (µm)	536.1 ± 28.4 µm	542.5 ± 32.9 µm	**0.042**
Mean Hexagonality (%)	42.55 ± 9.3	43.18 ± 7.3	0.073
DME (n = 10 eyes) Mean age, 62.4 years			
Mean Cell Density (cells/mm^2)	2651.89 ± 315.7	2691.44 ± 268.7	0.369
Mean Central Corneal Thickness (µm)	541.22 ± 34.6	541.22 ± 34.6	0.217
Mean Hexagonality (%)	45.44 ± 7.0 %	42.33 ± 4.2 %	0.320
Pseudophakic (n = 22 eyes) Mean age, 77.7 years			
Mean Cell Density (cells/mm^2)	2321.18 ± 532.3	2137.36 ± 412.7	0.080
Mean Central Corneal Thickness (µm)	533.18 ± 28.4	540.55 ± 36.3	0.077
Phakic (n = 28 eyes) Mean age, 66.1 years			
Mean Cell Density (cells/mm^2)	2636.57 ± 347.1	2585.00 ± 373.9	0.509
Mean Central Corneal Thickness (µm)	541.75 ± 32.3	544.29 ± 30.5	0.475

Age-related macular degeneration; AMD, diabetic macular edema; DME

DISCUSSION

Vascular endothelial growth factor and its receptors were determined in corneal endothelial cells (12,14). In studies of animal models, it has been shown that intracameral anti-VEGF agents increase the apoptotic activity in the corneal endothelium (15-17). An experimental macaque study showed that intravitreal injection provided a sufficient amount of aflibercept in the anterior chamber (13). There are in vitro studies showing that anti-VEGF can disrupt the tight junctions of the RPE (9). With a similar mechanism, we thought that it might affect endothelial cell morphology by disrupting the tight junctions of the corneal endothelium. Particularly with aging, cornea endothelial cells and their tight junctions may be more susceptible (18). It is still not clear how aflibercept affects the corneal endothelium. In this study, we investigated the effect of aflibercept on the corneal endothelium.

Endothelial cells on the inner surface of the cornea provide structural integrity and transparency. Damaged endothelial cells are rarely regenerated (7). The mean

corneal endothelial CD is 3500–4000 cells/mm^2 in children, 2700–3000 cells/mm^2 in adults, and 2500 cells/mm^2 in elderly individuals. The endothelial CD gradually decreases with age. With aging, the quantitative decrease is 0.5% per year. Clinically significant corneal edema develops when the endothelial CD is less than 700 cells/mm (19,20). In this study, the mean endothelial CD was 2565.0 ± 461.3 cells/mm^2 at pre-injection and 2500 ± 447.7 cells/mm^2 at post-injection. Although the endothelial CD was decreased, the value was not statistically significant. In patients with exudative AMD, the mean CD decreased from 2439.71 ± 476.16 cells/mm^2 to 2286.18 ± 427.24 cells/mm^2 after intravitreal aflibercept injection, but this difference was not statistically significant. Nevertheless, in AMD patients, the CCT was increased from 536.11 ± 28.4 µm to 542.45 ± 32.9 µm, which was statistically significant (p = 0.042). The decrease of CD was correlated with the increases of CCT. In AMD patients, the mean age was older than the DME patients, respectively 73.1 and 62.4 years. Additionally 61.1% of 36 eyes were pseudophakic in AMD group. Pre-injection and post-injection CD values were lower in AMD patients than DME patients because of having older age and high frequency of pseudophakia. Besides, the CD values did not decrease in the DME group after the three aflibercept injections, whereas they decreased in the AMD group. Decreasing endothelial cells with age may be more susceptible to aflibercept injection and the transient increase in the IOP after the injection. Previous studies have shown that, after intravitreal anti-VEGF injections, there was an acute and transient IOP increasing (8,21,22). In this study, there was no significant difference in IOP values before the first and after the third aflibercept injection. However, we did not measure IOP within early hours after the injection. After the intravitreal injection, intraocular inflammation and IOP elevation may cause CCT changes due to impairment of endothelial cell pump function and direct effects of corneal mechanical properties (23). In the study of Atılgan et al (24), there was no significant change in CD, CoV and Hex values after aflibercept injection in AMD patients, but the corneal densitometry parameters in the posterior layer were statistically higher after the loading dose. They speculated that anti-VEGF agents could increase corneal densitometry by reducing corneal tight junctions and may lead to slightly reduction in corneal transparency.

Park et al (25) suggested that endothelial CD did not change after intravitreal bevacizumab treatment. Guzel et al (26) reported that intravitreal ranibizumab and bevacizumab had not any devastating effect on corneal endothelium. Guler et al (27) showed that dexamethasone implants had affected corneal endothelial cells. They also showed a temporary decrease in endothelial CD, but no change in cell morphology. Chiang et al (28) showed that intravitreal bevacizumab injection did not change corneal endothelial cells at six months after the injection. Lass et al (29) reported that repeated injections of aflibercept over 52 weeks did not cause corneal endothelial cell toxicity. Muto et al (30) measured CD and corneal morphology before and after intravitreal aflibercept injections in DME and retinal vein occlusion patients. Only the maximum cell size varied significantly six months after injection, but the mean number of injections (1.43 times / 6 months) was lower than in our study (3 times / 3 months). In contrast to previous studies, in the study by Arslan et al (31) one month after the first and second injection of intravitreal anti-VEGF agents, CD was lower in phakic eyes than pre-injection values. There was no significant difference in the pseudophakic eyes. They speculated that phacoemulsification had already decreased the CD and, therefore, the CD values in pseudophakic eyes did not decrease following intravitreal injections. In our study, the CD values were similar between phakic and pseudophakic eyes.

The coefficient of variation is the standard deviation of the cell area and Hex is the percentage of cells with six sides. The mean endothelial CoV was 27% and Hex was 60%–80% in healthy young adults. Coefficient of variation and Hex increase with age if endothelial cells are damaged, Hex is expected to decrease (18,30). In our study, the mean endothelial CoV was 45.0 ± 6.2 % at pre-injection and 46.0 ± 8.7 % after three injections. Hexagonality was 42.5 ± 8.7 % at pre-injection and 44.5 ± 6.7 % after three injections. When compared with previous studies, we found higher CoV and lower Hex values (27,29,30). The difference in CoV and Hex values from the literature reports may be related to the older age of our patients and higher frequency of psedophacia in our study. However, there was no statistically significant difference in the corneal morphological measurements before and after the injections.

LIMITATIONS

The limitations of this study were retrospective course, relatively small sample size and short follow-up time.

CONCLUSION

In conclusion, intravitreal aflibercept injections did not have any damaging effect on the corneal endothelium. However, prospective clinical studies are required to confirm the effects of intravitreal aflibercept injections on the corneal endothelium.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The study was approved by the hospital (Bursa Yuksek Specialization Training and Research Hospital) ethics committee (2011-KAEK-25 2019/02-11).

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